

Original article

A prospective, randomized, double-blind, placebo-controlled multi-centre study on the efficacy and safety of sublingual immunotherapy (SLIT) in children with seasonal allergic rhinoconjunctivitis to grass pollen

Background: Especially in childhood, sublingual immunotherapy (SLIT) could offer advantages over subcutaneous therapy. However, limited data on its efficacy is available.

Methods: In four German centres 97 children (age 3–14 years) with allergic rhinoconjunctivitis to grass pollen were enrolled in a prospective, double-blind trial comparing SLIT (Pangramin SLIT®; ALK-SCHERAX, 0.5 µg major allergens, three times per week, 32 months) with placebo. Primary endpoint was a multiple symptom-medication score for changes in seasonal diary entries between the first and third year of the study (SLIT $n = 39$; placebo $n = 38$).

Results: The multiple symptom-medication score was significantly reduced by SLIT to 77.3% of the placebo group ($P = 0.0498$). The subsequent analysis of the single endpoints did not reveal significant differences for symptom scores in favour of SLIT (85.1% of placebo group; $P = 0.22$). However, the medication score improved significantly (67.1% of placebo group; $P = 0.0025$). Furthermore, secondary endpoints assessing *in vivo* immune responses did not differ significantly between the groups. However, retrospective analysis showed some inhomogeneity for clinical and *in vitro* parameters at the beginning of the study. Allergic side effects with possible relation to the study drug were reported in both groups (SLIT 49%, placebo 27%, $P = 0.026$).

Conclusion: Our study indicates that SLIT had a positive effect on the reduction of a multiple symptom-medication score, mainly by significantly reducing rescue medication use, but had no significant effect on symptoms alone in children with rhinoconjunctivitis to grass pollen compared with a placebo.

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Key words: children; conjunctival provocation test; grass pollen; immunoglobulin E; immunoglobulin G; rye pollen; skin prick test; specific immunotherapy; sublingual immunotherapy.

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Seasonal allergic rhinoconjunctivitis (SAR) has a prevalence of approximately 15% in children up to 7 years of age; of these, 20% develop asthma later in life and 11–73% show bronchial hyper-responsiveness (1). Pharmacotherapy, which is often effective, only ameliorates symptoms. Subcutaneous specific immunotherapy (SIT) is a potentially curative approach by altering immune

responses (2). The initial investigation of reduction of symptoms in grass pollen allergic patients by immunization with grass pollen extract was published almost a century ago (3). Since then it has been shown in well-controlled studies that subcutaneous SIT is a beneficial therapy for SAR and guidelines for its usage have been developed (4, 5).

Recent studies indicate that SIT can prevent the development of new sensitizations (6–8). Furthermore, it has been established that SIT is able to prevent the development of asthma in children with SAR (7). For several years, considerable efforts have been made to overcome the disadvantages of subcutaneous SIT by establishing alternative routes of application. Sublingual immunotherapy (SLIT) seems to offer practical advan-

Abbreviations: BU, biological unit; CPT, conjunctival provocation test; ECP, eosinophil cationic protein; HEP, histamine equivalent in skin prick test; NO, nitric oxide; NPT, nasal provocation test; SAR, seasonal allergic rhinoconjunctivitis; SCORAD, Score of atopic dermatitis; SIT, specific (subcutaneous) immunotherapy; SLIT, sublingual immunotherapy; SPT, skin prick test; STU, specific treatment units.

tages for treatment and compliance because injections can be avoided, the treatment is generally well tolerated and the risk of severe allergic reactions seems to be negligible (9–11), making it especially attractive in children.

The published placebo-controlled, double-blind clinical studies with SLIT have recently been reviewed (9, 11) in a Cochrane analysis (10). Seven SLIT studies have been performed with grass pollen preparations in children and adults with SAR with and without asthma using various doses and durations (12–18). In three of these studies statistically significant reductions in symptom and medication scores were observed between SLIT and placebo (15, 17, 18). In one study no significant difference between SLIT and placebo was found in rhinitis symptom scores, but there was a significant reduction of the medication scores (12). Three studies showed no significant reduction in either symptom scores or medication scores (13, 14, 16). More studies are therefore required to evaluate the efficacy of this kind of treatment (9).

We therefore performed a randomized long-term, double-blind, placebo-controlled multi-centre study to investigate the efficacy and safety of SLIT in children with grass pollen allergic SAR.

Material and methods

Study design

We performed a prospective, randomized, double-blind, placebo-controlled multi-centre study to investigate the efficacy and safety of SLIT in children with SAR to grass and/or rye pollen and with or without seasonal asthma. Children were recruited by the pediatric departments of four German university hospitals (Berlin, Freiburg, Dresden, Munich) and treated with SLIT or placebo for 32 months from January 1999 to November 2001 (Fig. 1).

Primary endpoint was a multiple symptom-medication score for changes in seasonal diary entries between the pollen season of the third year (G3) and the first year with SLIT (G1). The score was defined as the rank sum of symptom scores at eyes, nose and lung and of a medication score. In a subanalysis, symptom scores at eyes, nose and lung and the medication score were tested separately as a closed test procedure. Symptoms (eyes 3 items, nose 4, lung 4; score 0–3) and medication (seven rescue drugs, score 0–1) were documented daily for the 6 weeks of grass pollen season with maximum pollen count in each trial centre. Due to differences for pollen exposure in the four centres, located in different geographical regions of Germany, scores were adjusted to a pollen count of 1000 pollen grains/m³ for 6 weeks of the grass pollen season.

Secondary parameters were the assessment of titrated skin prick tests (SPTs), conjunctival provocation tests (CPTs), nasal provocation tests, lung function tests, exhaled air nitric oxide (NO) concentrations, dermatological status according to the Score of Atopic Dermatitis (SCORAD), total and specific immunoglobulin E (IgE) and immunoglobulin G₄ (IgG₄), and eosinophil cationic protein (ECP) in nasal secretions. The study was approved by all local ethics committees and written consent of the participants and/or their parents obtained. The study was performed according to ICH-GCP guidelines.

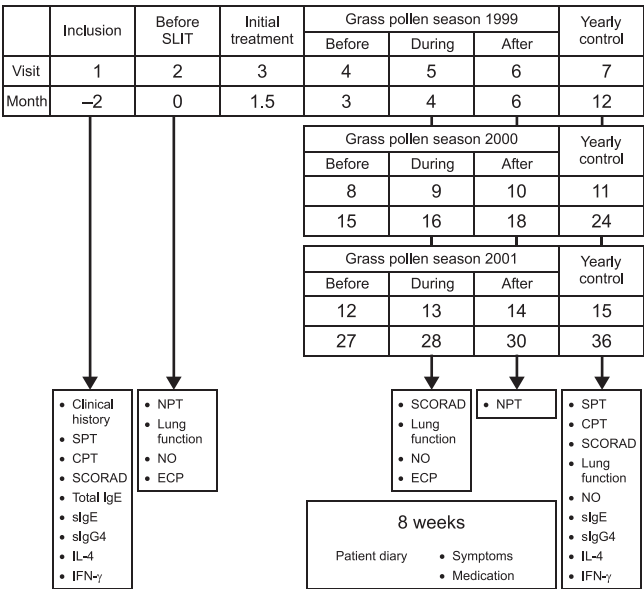


Figure 1. Flow chart of study conduct. Times of visits and investigated endpoints during the study.

Patients

We studied 97 children aged 3–14 years with seasonal allergic rhinitis with specific IgE to grass pollen (CAP-class ≥ 2), a wheal size of ≥ 3 mm or a skin index of ≥ 0.6) in SPT, and a positive CPT at a specific allergen concentration $\leq 100\,000$ SQ-U/ml. Exclusion criteria were perennial asthma with a need for permanent topical or systemic corticosteroid treatment, atopic dermatitis with a need for permanent treatment with corticosteroids, systemic treatment with corticosteroids or immune suppressive drugs within the 4 weeks before the start of the study, perennial allergic rhinitis, treatment with SIT within the 3 years before the start of the study and the known contraindications of SIT according to the EAACI position paper (19). Patients were randomized into the two treatment arms (placebo-verum ratio 1:1) according to the strata age and bronchial asthma (group A: 3–6 years, group B: 7–14 years; group 1: no seasonal allergic asthma; group 2: existing bronchial seasonal asthma) in consecutive order at inclusion.

SLIT

Patients in the active group were treated with standardized allergens from a 5-grass mixture composed of *Dactylis glomerata*, *Festuca pratensis*, *Lolium perenne*, *Phleum pratense* and *Poa pratensis* in equal parts suspended in physiologic saline solution with 50% glycerol and 0.3% phenol (Pangramin SLIT[®]; ALK-SCHERAX, Hamburg, Germany). The potency was expressed in specific treatment units (STU); 1000 STU were equivalent to 25 biological units (BU) and contained 2.5 µg of major grass pollen allergens. The monthly dose during maintenance treatment was 6 µg (0.5 µg/dose, 3 times/week). The median for the total duration of treatment was 32 months (January 1999 to November 2001) with a median cumulated dose of 188 µg allergens.

Children were instructed to apply the relating amount of the SLIT preparation under the tongue and to wait for 3 min before swallowing. Initial treatment of SLIT was applied from five vials (0–4) with the concentrations 1.6, 8, 40, 200 and 1000 STU/ml

beginning with one drop from vial 0 taken on days 1 and 2 and continued by two drops on days 2 and 3 until reaching five drops on days 9 and 10. The dose was then increased by 1 drop/day from one to five drops from vials 1–4. Total duration of the initial treatment was 4 weeks. The dose of five drops from vial 4 was applied as a maintenance dose three times a week until the end of the 32 months treatment period. Placebo vials contained the Pangramin solution without allergen. Patients and parents were instructed for correct application of SLIT at inclusion. Diaries were checked at the visits indicated in Fig. 1 for the compliance for administration of SLIT.

Assessment of symptoms and rescue medication use

For the pollen season patients were asked to record daily their clinical symptoms and rescue medication. Symptoms were recorded on a scale 0–3 (0, no symptoms; 1, weak symptoms; 2, moderate symptoms; 3, severe symptoms) for ocular symptoms (itching/tear flow/reddening), nasal symptoms (obstruction/itching/rhinorrhea/sneezing) and bronchial symptoms (tightness of chest/wheezing/cough/dyspnea). The average weekly scores from the 6 weeks of the season with local peak pollen count were evaluated.

As rescue medication DSCG eye drops, nasal decongestants (tramazoline), topical antihistamines (levocabastine eye drops and nasal spray), systemic antihistamines (cetirizine) and intranasal corticosteroids (fluticasone) were supplied for symptomatic treatment, with β -sympathomimetics (salbutamol) and inhalative corticosteroids (budesonide) for treatment of bronchial symptoms. The use of the seven rescue drugs was scored as one point for every type of drug per day of application, and the weekly average scores for the daily sum of points of the 6 weeks with peak pollen count in grass pollen season were evaluated.

Total IgE, specific IgE and IgG₄

Serum samples were analysed for total IgE, specific grass pollen IgE and IgG₄ by a fluorescence enzyme immunoassay (CAP-RAST; Pharmacia-Diagnostics, Freiburg, Germany) according to the manufacturer's instructions.

Skin prick test

Allergic sensitization was further analysed by SPT responses (ALK-prick SQ; ALK-SCHERAX, Hamburg, Germany) at the visits shown in Fig. 1. Concentrations of 1, 3 and 10 histamine equivalents (HEP; 10 HEP = 10 000 BUs/ml, dissolved in 50% glycerol) were used. A 0.9% saline solution was used as negative control and 1% histamine dihydrochloride as positive control. The area of wheal and erythema caused by allergens and positive control were determined planimetrically and expressed as skin index (SI: wheal area related to area for the positive control). The mean dose of allergen necessary to generate SI = 1 was derived from the three concentrations tested and was evaluated as threshold dose.

Conjunctival provocation test

A drop of test solution (ALK-lyophilisiert SQ grasses; ALK-SCHERAX, Hamburg, Germany) was applied into the lower conjunctival sac alternating between the two eyes starting with 0.9% sodium chloride as negative control. Allergen concentrations were increased in half-log₁₀ steps from 100 to 100 000 SQ-U/ml. A positive reaction was recorded when redness of the conjunctiva and itching occurred.

Nasal provocation test

Test solutions (negative control: ALK-diluent; allergen preparations: ALK-lyophilisiert SQ grasses, ALK-SCHERAX, Hamburg, Germany) were applied to the inferior turbinate by a nasal spray applicator at increasing doses concentrations from 10 to 100 000 SQ-U/ml at 10 min intervals. A positive result was defined as a total symptom score of more than 3 points for rhinorrhea (0, no secretion; 1, little secretion; 2, much secretion; 3, very much secretion), irritation (0, 0–2 sneezes; 1, 3–5 sneezes; 2, more than 5 sneezes) and remote symptoms (0, no; 1, tear flow, and/or palatal itching and/or itching of the ears; 2, conjunctivitis, and/or chest tightness, and/or wheezing, and/or shortness of breath).

Lung function measurements

Lung function measurements were performed by spirometry as far as feasible by age and compliance of the children using a bodyplethysmograph (E. Jaeger, Wuerzburg, Germany), and the inspired vital capacity (IVC), forced vital capacity (FVC), forced expiratory volume in one second (FEV₁), maximum expiratory flow (PEF) and PEF at 50% vital capacity (MEF₅₀) were evaluated at several visits before and within the pollen seasons (Fig. 1).

Exhaled nitric oxide concentrations

Exhaled NO-concentrations were measured in the subgroup of patients from the study centre Freiburg during a single-breath exhalation by means of a computerized system (Exhaled Breath Analyser; Aerocrine AB, Stockholm, Sweden) with a chemiluminescence analyser (CLD 77AM; Eco Physics, Duernten, Switzerland) as described previously (20). In brief, two-point calibrations (80 ppm NO balanced with N₂) and NO zeroing (NO-free air) were set before the measurements. Children inhaled NO-free air to avoid contamination with ambient NO, and performed a slow vital capacity manoeuvre with a target flow of 70 ml/s measured by a heated pneumotachograph (Hans Rudolph, Kansas City, KS, USA). A fixed expiratory resistance with approximately 100 cmH₂O/l/s was used to increase oral pressure, to prevent contamination with NO from the nasopharynx. Exhaled air was led via a non-rebreathing valve into a Teflon tubing system connected to the analyser and continuously sampled from the exhalation limb of the system. After a 15 to 20 s period of exhalation, mean NO values were calculated from the 60 to 90% part (plateau level) of the exhalation.

SCORAD

Severity of eczema was scored according to the SCORAD-score (21), with assessment of 'topography' items (affected skin area), 'intensity' criteria (extent of erythema, oedema, crusts, excoriations, lichenification, xerosis) and subjective parameters (extent of itch and loss of sleep). The maximum possible score was 103 points.

Eosinophil cationic protein in nasal lavage fluid

For nasal lavage, 4 ml of a sterile phosphate-buffer solution (37°C) was instilled into one nostril. After keeping the solution in the throat for 10 s, which had been closed by a breathing manoeuvre, the recovery was collected in a sterile cup. The procedure was repeated on the contra-lateral side. After incubation at room temperature for 1 h, the recovery was centrifuged at 800 g and cell-free supernatants frozen at –80°C. ECP concentrations were determined

in duplicate samples by radio immunoassay (ECP-RIA; Pharmacia) according to the manufacturer's instructions. All nasal lavages were performed at the same time in the afternoon (± 2 h).

Safety

The safety analysis was based on the documentation of symptoms and adverse events by patients (diaries) and doctors (each visit) and their global assessment of tolerability at the end of the study.

Sample size and statistics

The sample size was determined on the basis of previously published results for symptom-medication-scores. Using an expected standardized difference of 0.5, a significance level of $\alpha = 0.05$ (one-tailed) and a power of $1 - \beta = 0.8$, at least 50 patients per treatment arm should complete the third grass pollen season (G3). The changes of the pollen count adjusted symptom and medication scores in 2001 compared with 1999 were analysed as multiple endpoint by means of O'Brien's non-parametric procedure (22, 23). The components of the O'Brien rank sum (symptom scores at eyes, nose and lung and the medication score) were tested separately in a closed test procedure controlling the multiple type I error. The SLIT and placebo group were compared using the Mann-Whitney-Wilcoxon *U*-test. For intra-group comparisons of the seasons 1999 and 2001 the Wilcoxon signed rank test was used.

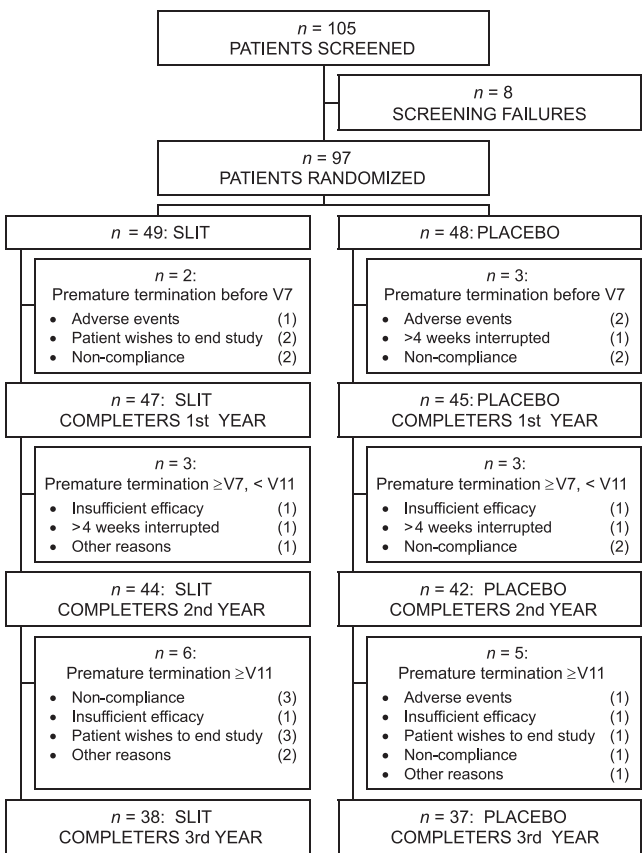
The further endpoints were compared among treatment groups by means of the *U*-test, *t*-test and analysis of covariance (ANCOVA) for the repeated measurement design. Logarithmically transformed data was analysed with regard to IgE and specific IgG₄. The χ^2 -test was used for categorical data. Due to an interim analysis after 2 years of treatment, and in accordance with the ICH-E9 document (Statistical principles for Clinical Trials) a *P*-value < 0.05 (two-tailed) was regarded as statistically significant.

Results

Patients

Ninety-seven children with SAR to grass pollen were included in the study. The numbers of patients continuing the study at the various follow-up visits are shown in Fig. 2. Thirty-one patients were enrolled in Berlin, 26 in Freiburg, 20 in Dresden and 20 in Munich. Diaries with daily assessments of symptoms and medication use (full analysis set) could be evaluated from 39 patients with SLIT and 38 patients with placebo for the grass pollen seasons in the third year (G3) and in the first year (G1) after starting SLIT. Thirty-eight patients with SLIT and 37 patients with placebo completed 32 months of treatment. Accordingly, the number of 50 patients per treatment arm completing the third season as statistically planned for the sample size could not be reached.

The baseline data are shown in Table 1. Homogeneity tests indicated that the SLIT and placebo groups were inhomogeneous at the start of the study despite randomization (for age and history of asthma). Baseline values of total and specific IgE and IgG₄ were significantly higher in the SLIT group. Patients with atopic dermatitis in the



(Multiple entries of premature termination)

Figure 2. Numbers of study participants during the study.

SLIT group had significantly larger areas of affected skin, although this was not reflected by the SCORAD scores. Inhomogeneity ($P < 0.150$) was also between the groups for ocular symptom scores ($P = 0.0998$) and medication scores ($P = 0.0215$) during the first pollen season (G1, Table 2).

Symptom and medication scores

Diaries were available for evaluations for the first (G1) and the third grass pollen season (G3) from 39 participants of the SLIT group and 37 of the control group. The mean symptom and medication scores adjusted to 1000 pollen grains/m³/6 weeks of the first grass pollen season (G1) and of the third grass pollen season (G3) are shown in Table 2. Treatment with SLIT did not reduce ocular, nasal nor lung symptoms significantly over 3 years of therapy compared with placebo treatment (combined symptom score 85.1% of placebo group). However, the medication score improved significantly in the SLIT group compared with placebo (67.1% of placebo group).

The simultaneous analysis (ENBM) of symptoms at eyes (E), nose (N), bronchi (B) and medication (M)

Table 1. Baseline characterization

	SLIT	Placebo	P-value
Total number of patients	49	48	
Age (n, %)			0.948
3–6 Years, without seasonal asthma	7 (14.3)	9 (18.8)	
3–6 Years, with seasonal asthma	2 (4.1)	2 (4.2)	
7–14 Years, without seasonal asthma	22 (44.9)	20 (41.7)	
7–14 Years, with seasonal asthma	18 (36.7)	17 (35.4)	
Gender (n, %)			0.221
Male	30 (61.2)	35 (72.9)	
Female	19 (38.8)	13 (27.1)	
Retrospective symptoms in the prestudy season (mean $\pm$ SD)			
Ocular score (range 0–9)	5.63 $\pm$ 2.54	4.79 $\pm$ 2.63	0.112
Nasal score (range 0–12)	5.69 $\pm$ 2.46	5.50 $\pm$ 2.26	0.687
Bronchial score (range 0–12)	2.35 $\pm$ 2.70	2.50 $\pm$ 2.95	0.790
Total score (range 0–33)*	13.67 $\pm$ 4.88	12.79 $\pm$ 4.82	0.373
Atopic dermatitis (n, %)	11 (22.4)	6 (12.5)	0.198
SCORAD, median score	15.40	20.60	0.521
% Affected skin	9.14 $\pm$ 7.71	20.50 $\pm$ 11.88	0.030
SPT, median threshold dose (HEP)	2.44	2.38	0.928
CPT, geometric mean of dose (SQ-U/ml)	3838	4446	0.629
NPT, geometric mean last dose (SQ-U/ml)*	12649	14798	0.685
Total IgE, geometric mean (kU/l)	322.8	212.8	0.055
Specific IgE, geometric mean (kU/l)	42.46	29.90	0.111
Specific IgG ₄ , geometric mean (μ g/l)	418.3	144.4	0.034
ECP, median (ng/ml)*	6.53	5.99	0.203

Unless indicated: figures from inclusion visit.

* At end of run-in phase.

$P < 0.150$: inhomogeneity assumed.

revealed a reduction of the score in favour of SLIT (77.3% of placebo group), which was significant (Fig. 3). Other combinations of simultaneous tests were not significant as long as lung symptoms were included in the multiple analysis. A significant comparable effect was found for the simultaneous analysis of symptoms at eyes, nose and medication (ENM), eyes and medication (EM) and nose and medication (NM) (Fig. 3).

Scores were reduced by 33.2% in the SLIT group and 2.3% in the placebo group for ocular symptoms, 29.3 and 18.7%, respectively for nasal symptoms, 36.4 and 36.0% for bronchial symptoms, and 57.7 and 10.7% for medication. For these single endpoints the reductions within the placebo group (G3 vs. G1) were not significant,

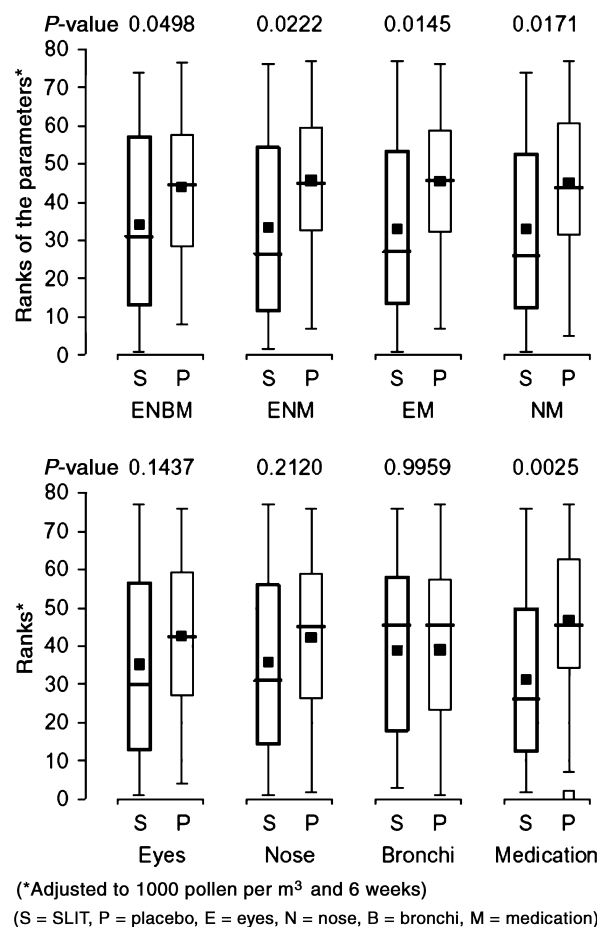


Figure 3. Results of the multiple analysis of the differences between the third (G3) and the first grass pollen season (G1) for ocular, nasal and lung symptom scores and medication scores in various combinations.

whereas in the SLIT group ocular and nasal symptoms improved ($P = 0.026$; $P = 0.010$), as well as the medication score ($P < 0.001$).

In-vitro tests

The results of serum analyses for specific IgE and IgG₄ are shown in Table 3. Neither the changes in specific IgE

Table 2. Adjusted symptom and medication scores for 6 weeks with peak pollen counts in the first and third grass pollen season

Mean score (SD)	SLIT (n = 39)			Placebo (n = 38)			SLIT vs Placebo (P-value*)
	G1	G3	Change G3 vs G1	G1	G3	Change G3 vs G1	
Eyes	10.22 (11.78)	6.83 (13.63)	-3.39 (12.36)	5.27 (6.29)	5.15 (9.81)	-0.12 (7.80)	0.1437
Nose	19.39 (18.25)	13.71 (23.12)	-5.68 (22.13)	15.57 (19.03)	12.66 (21.65)	-2.91 (17.39)	0.2120
Bronchi	4.01 (6.75)	2.54 (5.00)	-1.46 (4.18)	3.92 (7.13)	2.50 (5.06)	-1.41 (5.31)	0.9959
Medication	6.00 (5.80)	2.54 (3.58)	-3.46 (4.75)	3.18 (3.99)	2.85 (3.87)	-0.34 (5.06)	0.0025

*U-test.

G1, first grass pollen season; G3, third grass pollen season.

Standard deviation are given in parenthesis.

Table 3. Specific IgE and specific IgG₄ at postseasonal visits

	V1	V7	V11	V15	change V15 vs V1 (%)	SLIT vs Placebo (<i>P</i> -value*)
slgE (kU/l)						
SLIT (<i>n</i> = 37)	48.97	60.14	56.37	79.83	+63.0	
Placebo (<i>n</i> = 36)	27.32	39.25	39.19	60.97	+123.1	0.0734
slgG ₄ (μg/l)						
SLIT (<i>n</i> = 37)	465.6	573.0	563.9	734.6	+57.8	
Placebo (<i>n</i> = 36)	102.3	188.7	142.4	249.5	+144.0	0.2310

slgE, slgG₄, geometric mean.

V1, at inclusion; V7, V11, V15, after first, second, third year of treatment.

**t*-test.

after 3 years SLIT (V15) vs baseline nor for those of IgG₄ differed between treatment groups.

In-vivo tests

The results for SPT, CPT and NPT are shown in Table 4 and 5 and did not reveal significant differences. The results of the lung function measurements at the different time points for the patients of the full analysis set (SLIT *n* = 37, placebo *n* = 31) showed the expected significant age-dependent increase. FEV₁ increased from the adjusted baseline value of 2.12–2.88 L with SLIT and to 2.75 L with placebo (SLIT vs placebo: *P* = 0.1132). A slight but

Table 4. Skin prick tests (SPT) and the conjunctival provocation tests (CPT) at post-seasonal visits

	V1	V7	V11	V15	change V15 vs V1	SLIT vs Placebo <i>P</i> -value
SPT						
SLIT (<i>n</i> = 38)	2.56	1.76	1.54	2.70	+0.02	
Placebo (<i>n</i> = 34)	2.31	1.94	3.25	6.63	+0.70	0.1740*
CPT						
SLIT (<i>n</i> = 38)	3590	5011	5657	7659	+113.3%	
Placebo (<i>n</i> = 35)	4713	4876	6563	5952	+26.3%	0.2335†

SPT, median of the threshold dose (HEP); CPT, geometric mean of the dose (SQ-U/ml).

V1, at inclusion; V7, V11, V15, after first, second, third year of treatment.

**U*-test; †*t*-test.

Table 5. Nasal provocation tests (NPT) at visits at the end of the pollen season

	V2	V6	V10	V14	change V14 vs V2 (%)	SLIT vs Placebo (<i>P</i> -value*)
NPT						
SLIT (<i>n</i> = 23)	14925	12217	36747	24621	+65.0	
Placebo (<i>n</i> = 21)	21544	15505	64495	51795	+140.4	0.6106

NPT, geometric mean of patients with positive test at V2.

V2, before treatment; V6, V10, V14, after first, second, third grass pollen season.

**t*-test.

significant difference between the groups was seen only at V9 during the pollen season in the second year (SLIT: 2.51 L, placebo: 2.32 L; *P* = 0.0206). The PEF (l/s) changed from an adjusted baseline value of 4.56 to 5.90 l/s with SLIT and 5.63 l/s with placebo (SLIT vs placebo: *P* = 0.1916). MEF₅₀ increased from an adjusted baseline value of 2.70 to 3.49 l/s with SLIT and 3.27 l/s with placebo (SLIT vs placebo: *P* = 0.1203).

The adjusted NO baseline value for the subgroup of 16 patients was 17.99 ppb. In the first grass pollen season, NO concentrations were 52.36 ppb (SLIT) and 49.31 ppb (placebo); values for the second season were 45.82 ppb (SLIT) and 40.34 ppb (placebo), and in the third season 42.94 ppb (SLIT) and 44.35 ppb (placebo) were measured. Differences between the SLIT and the placebo group were not significant for the three grass pollen seasons (ANCOVA, *P* = 0.7277).

The secretion of ECP into nasal lavage fluids for the three grass pollen seasons during SLIT could be analysed for 31 patients with SLIT and 25 patients with placebo treatment. The adjusted baseline value was 6.83 ng/ml before SLIT. ECP levels during the pollen seasons increased to 34.45 ng/ml (SLIT) and 18.30 ng/ml (placebo) in the first season, 32.65 ng/ml (SLIT) and 13.83 ng/ml (placebo) in the second season and 23.73 ng/ml (SLIT) and 13.41 ng/ml (placebo) in the third season. ECP values were significantly higher in the SLIT group than in the placebo group for all of the three grass pollen seasons (ANCOVA, *P* = 0.0127). In the third grass pollen season (G3) compared with the first grass pollen season ECP levels were reduced by the SLIT treatment by 31.1% and by 26.7% with placebo treatment (*P* = 0.6583; *U*-test).

Twenty-four of the 38 children in the SLIT-group, and 30 of 37 in the placebo group who continued the study up to the final investigation had a negative SCORAD; 14 children in the SLIT group and seven in the placebo group had signs of atopic dermatitis at least once. For these patients the development of the dermatological status during treatment could be evaluated. The adjusted mean of the baseline SCORAD was 11.61. The SCORAD means during the visits at peak pollen seasons were 7.47, 10.46 and 9.80 for the SLIT group and 8.46, 4.29 and 11.00 for the placebo group, respectively, but no significant differences were observed between SLIT and placebo throughout the different times (peak season scores, out of season scores) of assessments (ANCOVA, *P* = 0.5676).

Safety

Allergic reactions at least possibly related to the trial medication occurred in 24 of 49 (49.0%) patients with SLIT and 13 of 48 (27.1%) placebo-treated patients (*P* = 0.026). Predominantly local reactions (30.6% vs 18.8%) and nasal complaints (14.3% vs 6.3%) were reported. Other adverse events with at least possible

association to treatment were equally distributed (12.2% vs 12.5%). Termination of the study due to adverse events was registered in one patient in the active and three children in the placebo group. In one actively treated patient an acute exacerbation of bronchial asthma possibly related to the trial medication necessitated hospitalization and has, therefore, been classified as serious. The global assessment of tolerability by patients and doctors after 2 and 3 years did not show significant differences.

Discussion

Our data indicate that a long-term treatment with grass pollen SLIT in children with SAR with and without asthma was able to significantly reduce the use of rescue medication, but had no significant effect on symptoms compared with placebo. In the multiple endpoint analysis, the combination of symptoms at eyes, nose, bronchi and medication and the combination of the single symptoms at eyes and nose with medication were significantly different in favour of SLIT, but not the single symptom scores without medication. When bronchial symptoms were included in the analysis no significant differences for the primary endpoint were observed between the third and the first grass pollen season during SLIT.

Other studies involving only children have also found no significant reduction in symptom or medication scores by SLIT, although the numbers of children in these four studies was small (total $n = 152$). Furthermore, different allergens (*Dermatophagoides pteronyssinus*, *Parietaria* and olive pollen) and doses have been used and therefore conclusions can only be drawn with caution (24–27). Concerning SLIT studies in adults, however, the recent Cochrane analysis of the published placebo-controlled studies has identified a significant reduction in both symptoms and medication scores, especially for seasonal allergens such as grass pollen (13, 14, 16, 18, 28) and *Parietaria* (26, 29).

Subcutaneous SIT studies resulted in a pooled reduction of approximately 60–75% for symptoms and for symptomatic medication use (2, 30, 31). In our study, SLIT was able to reduce the ocular symptom scores by 33%, the nasal symptom scores by 29%, and bronchial scores by 36% compared with 2, 19, and 36% in the placebo group, respectively with no significant difference between the groups. The medication scores were significantly reduced by 58% vs 11% by placebo. Studies comparing the efficacy of subcutaneous and SLIT are rare (28, 32) and it seems difficult to directly compare the studies due to various methodological differences (33).

One critical issue in the literature is the discussion of the allergen concentration and total allergen dose over the study period (33). In our study, the monthly dose (6 µg major grass pollen allergens) and the cumulative dose of

188 µg over 32 months was lower than in other studies with grass pollen SLIT. These have specified the doses of major allergens from 24 to 900 µg per month, or 84 to 16 200 µg over periods of 3.5 and 18 months (12, 14, 16, 18).

A positive correlation between allergen dose and efficacy of SIT has been documented for subcutaneous SIT, and seems likely for SLIT as well. However, there is no evidence from these and other studies with SLIT that a higher single dose or cumulative dose is able to induce a higher efficacy of SLIT. The authors of the recent meta-analysis were not able to analyse dose-related data due to the wide range of doses and due to the difficulty of comparing the different preparations. The authors identified a significant benefit for studies of less than 6 months and more than 12 months, but not those in between this range – they assume that this is mainly due to the paucity of information (10). Therefore thorough dose–response studies are necessary and, furthermore, the optimum duration of treatment remains to be established (33).

SLIT is often claimed to be more acceptable for patients compliance, since frequent outpatient visits are not required (11). In our study, for various reasons, we had a dropout rate of 23 and 10% due to non-compliance over 3 years (Fig. 2). Another study with 2 years treatment with SLIT in children also had 19.5% drop outs (26), whereas two long-term SIT studies via the subcutaneous route only lost 7 and 9%, respectively (7, 34). We conclude that the infrequent control of treatment therefore might be a drawback to be considered.

The analysis of the secondary *in vitro* and *in vivo* endpoints showed no significant effects of any parameter estimating changes in the immune system. Other studies have found conflicting results concerning the decrease in specific serum IgE and increase in specific IgG₄ (12, 14, 16, 25). In our patients, higher levels of specific IgE were found in the placebo group than in the SLIT group, with no significant differences for IgG₄ after 32 months of treatment compared with baseline values.

No significant differences between SLIT and placebo groups were observed for the multiple *in-vivo* provocation tests such as SPT, CPT and NPT after 32 months of treatment compared with the baseline values, which is in agreement with most of the published studies (10). Furthermore, lung function tests, exhaled NO measurements during grass pollen seasons and the dermatological status assessed by SCORAD were not significantly different between the SLIT and placebo groups at the repeated visits. We know of no data in the literature on these endpoints.

Despite randomization, which was conducted for age and history of asthma, homogeneity tests revealed that the treatment groups were inhomogeneous with more severe conjunctivitis and higher symptomatic medication in the first pollen season in the SLIT group, which also had higher total and grass pollen specific IgE and IgG₄ at baseline. This might have been avoided by recording symptoms and drug use in diaries during a baseline

season and by performing randomization according to matched pairs (9). The effect on treatment results remains unclear. Since we observed a reduction of symptoms between the first and the last year of the study only within the SLIT group, but not within the placebo group, the larger extent of effects might well be associated with initially higher symptom scores. For secondary parameters especially the ECP nasal fluid concentrations seem to be affected by this inhomogeneity.

In conclusion, our study indicates that SLIT with 0.5 µg major grass pollen allergens 3 times/week for

3 years compared with placebo was safe, and led to a reduction of a multiple symptom-medication score in 3–14 year old children with SAR with or without bronchial asthma which was significant, based on significant improvements for the rescue medication use. However, no significant effects were observed for ocular, nasal and lung symptoms if analysed as a single endpoint or for *in vitro* and *in vivo* immunologic parameters. Before recommending SLIT as an alternative therapeutic option to SIT (1), more studies are warranted.

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